

WHAT IS CLAIMED IS:

1 1. An isolated infectious chimeric parainfluenza virus (PIV) comprising
2 a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase
3 protein (L), and a partial or complete PIV vector genome or antigenome combined with one
4 or more heterologous gene(s) or genome segment(s) encoding one or more antigenic
5 determinant(s) of one or more heterologous pathogen(s) to form a chimeric PIV genome or
6 antigenome.

1 2. The chimeric PIV of claim 1, wherein said one or more heterologous
2 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are added as
3 supernumerary gene(s) or genome segment(s) adjacent to or within a noncoding region of
4 the partial or complete PIV vector genome or antigenome.

1 3. The chimeric PIV of claim 1, wherein said one or more heterologous
2 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are substituted for
3 one or more counterpart gene(s) or genome segment(s) in a partial PIV vector genome or
4 antigenome.

1 4. The chimeric PIV of claim 1, wherein said one or more heterologous
2 pathogens is a heterologous PIV and said heterologous gene(s) or genome segment(s)
3 encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or fragment(s)
4 thereof.

1 5. The chimeric PIV of claim 1, wherein the vector genome or
2 antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the
3 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of
4 one or more heterologous PIV(s).

1 6. The chimeric PIV of claim 5, wherein said one or more heterologous
2 PIV(s) is/are selected from HPIV1, HPIV2, or HPIV3.

1 7. The chimeric PIV of claim 5, wherein the vector genome or
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous HPIV(s).

1 8. The chimeric PIV of claim 7, wherein the vector genome or
2 antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous HPIV(s).

1 9. The chimeric PIV of claim 8, wherein one or more gene(s) or genome
2 segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from HPIV1
3 HN and F glycoproteins and antigenic domains, fragments and epitopes thereof is/are added
4 to or substituted within the partial or complete HPIV3 genome or antigenome.

1 10. The chimeric PIV of claim 8, wherein the vector genome or
2 antigenome is a partial or complete HPIV3 JS genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous HPIV(s).

1 11. The chimeric PIV of claim 10, wherein one or more gene(s) or
2 genome segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from
3 HPIV1 HN and F glycoproteins and antigenic domains, fragments and epitopes thereof is/are
4 added to or substituted within the partial or complete HPIV3 JS genome or antigenome.

1 12. The chimeric PIV of claim 9, wherein both HPIV1 genes encoding
2 HN and F glycoproteins are substituted for counterpart HPIV3 HN and F genes in a partial
3 HPIV3 vector genome or antigenome.

1 13. The chimeric PIV of claim 9, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 present within PIV3 JS *cp45* selected from mutations specifying an amino acid substitution
4 in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in
5 the N protein at a position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C
6 protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide substitution a 3' leader
7 sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of
8 JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to
9 nucleotide 62 of JS *cp45*

1 14. The chimeric PIV of claim 8, wherein one or more gene(s) or genome
2 segment(s) encoding one or more antigenic determinant(s) of HPIV2 is/are added to or
3 incorporated within the partial or complete HPIV3 genome or antigenome.

1 15. The chimeric PIV of claim 14, wherein one or more HPIV2 gene(s) or
2 genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic
3 domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial
4 or complete HPIV3 vector genome or antigenome.

1 16. The chimeric PIV of claim 6, wherein a plurality of heterologous
2 genes or genome segments encoding antigenic determinants of multiple heterologous PIVs
3 are added to or incorporated within the partial or complete HPIV vector genome or
4 antigenome.

1 17. The chimeric PIV of claim 16, wherein said plurality of heterologous
2 genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 are
3 added to or incorporated within a partial or complete HPIV3 vector genome or antigenome.

1 18. The chimeric PIV of claim 17, wherein one or more HPIV1 gene(s) or
2 genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic
3 domain(s), fragment(s) or epitope(s) thereof and one or more HPIV2 gene(s) or genome
4 segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic domain(s),
5 fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or
6 complete HPIV3 vector genome or antigenome.

1 19. The chimeric PIV of claim 18, wherein both HPIV1 genes encoding
2 HN and F glycoproteins are substituted for counterpart HPIV3 HN and F genes to form a
3 chimeric HPIV3-1 vector genome or antigenome which is further modified by addition or
4 incorporation of one or more gene(s) or gene segment(s) encoding one or more antigenic
5 determinant(s) of HPIV2.

1 20. The chimeric PIV of claim 19, wherein a transcription unit comprising
2 an open reading frame (ORF) of an HPIV2 HN gene is added to or incorporated within the
3 chimeric HPIV3-1 vector genome or antigenome.

1 21. The chimeric PIV of claim 20 selected from rPIV3-1.2HN, or rPIV3-
2 lcp45.2HN.

1 22. The chimeric PIV of claim 1, wherein the vector genome or
2 antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the
3 heterologous pathogen is selected from measles virus, subgroup A and subgroup B
4 respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2
5 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus,
6 Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza
7 viruses.

1 23. The chimeric PIV of claim 22, wherein said one or more heterologous
2 antigenic determinant(s) is/are selected from measles virus HA and F proteins, subgroup A
3 or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps virus HN and F
4 proteins, human papilloma virus L1 protein, type 1 or type 2 human immunodeficiency virus
5 gp160 protein, herpes simplex virus and cytomegalovirus gB, gC, gD, gE, gG, gH, gI, gJ,
6 gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus gp350 protein; filovirus
7 G protein, bunyavirus G protein, Flavivirus pre M, E, and NS1 proteins, and alphavirus E
8 protein, and antigenic domains, fragments and epitopes thereof.

1 24. The chimeric PIV of claim 22, wherein the vector genome or
2 antigenome is a partial or complete HPIV3 genome or antigenome or a chimeric HPIV
3 genome or antigenome comprising a partial or complete HPIV3 genome or antigenome
4 having one or more gene(s) or genome segment(s) encoding one or more antigenic
5 determinant(s) of a heterologous HPIV added or incorporated therein.

1 25. The chimeric PIV of claim 24, wherein the heterologous pathogen is
2 measles virus and the heterologous antigenic determinant(s) is/are selected from the measles
3 virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 26. The chimeric PIV of claim 25, wherein a transcription unit comprising
2 an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within
3 a HPIV3 vector genome or antigenome.

1 27. The chimeric PIV of claim 26 selected from rPIV3(HA HN-L),
2 rPIV3(HA N-P), rcp45L(HA N-P), rPIV3(HA P-M), or rcp45L(HA P-M).

1 28. The chimeric PIV of claim 24, wherein the vector genome or
2 antigenome is a chimeric HPIV genome or antigenome comprising a partial or complete
3 HPIV3 genome or antigenome having one or more gene(s) or genome segment(s) encoding
4 one or more antigenic determinant(s) of HPIV1 added or incorporated therein.

1 29. The chimeric PIV of claim 25, wherein the heterologous pathogen is
2 measles virus and the heterologous antigenic determinant(s) is/are selected from the measles
3 virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 30. The chimeric PIV of claim 29, wherein a transcription unit comprising
2 an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within
3 a HPIV3-1 vector genome or antigenome having both the HPIV3 HN and F ORFs
4 substituted by the HN and F ORFs of HPIV1.

1 31. The chimeric PIV of claim 30, selected from rPIV3-1 HA_{P-M} or
2 rPIV3-1 HA_{P-M} cp45L.

1 32. The chimeric PIV of claim 1, wherein the partial or complete PIV
2 vector genome or antigenome is combined with one or more supernumerary heterologous
3 gene(s) or genome segment(s) to form the chimeric PIV genome or antigenome.

1 33. The chimeric PIV of claim 32, wherein the vector genome or
2 antigenome is a partial or complete HPIV3 genome or antigenome and said one or more
3 supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN,
4 HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene
5 unit.

1 34. The chimeric PIV of claim 33, wherein one or both of the HPIV1 HN
2 and/or HPIV2 HN ORF(s) is/are inserted within the HPIV3 vector genome or antigenome,
3 respectively.

1 35. The chimeric PIV of claim 33, wherein the HPIV1 HN, HPIV2 HN,
2 and measles virus HA ORFs are inserted between the N/P, P/M, and HN/L genes,
3 respectively.

1 36. The chimeric PIV of claim 33, wherein the HPIV1 HN and HPIV2
2 HN genes are inserted between the N/P and P/M genes, respectively and a 3918-nt GU insert
3 is added between the HN and L genes.

1 37. The chimeric PIV of claim 33, which is selected from rHPIV3 1HN_{N-P},
2 P, rHPIV3 1HN_{P-M}, rHPIV3 2HN_{N-P}, rHPIV3 2HN_{P-M}, rHPIV3 1HN_{N-P} 2HN_{P-M}, rHPIV3
3 1HN_{N-P} 2HN_{P-M} HA_{HN-L}, and rHPIV3 1HN_{N-P} 2HN_{P-M} 3918GU_{HN-L}.

1 38. The chimeric PIV of claim 32, which contains protective antigens
2 from one, two, three or four pathogens.

1 39. The chimeric PIV of claim 32, which contains protective antigens
2 from one to four pathogens selected from HPIV3, HPIV1, HPIV2, and measles virus.

1 40. The chimeric PIV of claim 32, wherein said one or more
2 supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign
3 sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to
4 the wild-type HPIV3 genome length of 15,462 nt.

1 41. The chimeric PIV of claim 32, wherein the addition of said one or
2 more supernumerary heterologous gene(s) or genome segment(s) specifies an attenuation
3 phenotype of the chimeric PIV which exhibits at least a 10-to 100-fold decrease in
4 replication in the upper and/or lower respiratory tract.

1 42. The chimeric PIV of claim 1, wherein the vector genome or
2 antigenome is a human-bovine chimeric PIV genome or antigenome.

1 43. The chimeric PIV of claim 42, wherein the human-bovine chimeric
2 vector genome or antigenome is combined with one or more heterologous gene(s) or genome
3 segment(s) encoding one or more antigenic determinant(s) of a heterologous pathogen
4 selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses,
5 mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses,
6 herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses,
7 bunyaviruses, flaviviruses, alphaviruses and influenza viruses

1 44. The chimeric PIV of claim 42, wherein the vector genome or
2 antigenome comprises a partial or complete HPIV genome or antigenome combined with
3 one or more heterologous gene(s) or genome segment(s) from a BPIV.

1 45. The chimeric PIV of claim 44, wherein a transcription unit comprising
2 an open reading frame (ORF) of a BPIV3 N ORF is substituted in the vector genome or
3 antigenome for a corresponding N ORF of a HPIV3 vector genome.

1 46. The chimeric PIV of claim 45, wherein the vector genome or
2 antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

1 47. The chimeric PIV of claim 48, which is rHPIV3-N_B HA_{P-M}.

1 48. The chimeric PIV of claim 42, wherein the vector genome or
2 antigenome comprises a partial or complete BPIV genome or antigenome combined with one
3 or more heterologous gene(s) or genome segment(s) from a HPIV.

1 49. The chimeric PIV of claim 48, wherein one or more HPIV gene(s) or
2 genome segment(s) encoding HN and/or F glycoproteins or one or more immunogenic
3 domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial
4 or complete bovine genome or antigenome to form the vector genome or antigenome.

1 50. The chimeric PIV of claim 49, wherein both HPIV3 genes encoding
2 HN and F glycoproteins are substituted for corresponding BPIV3 HN and F genes to form
3 the vector genome or antigenome.

1 51. The chimeric PIV of claim 50, wherein the vector genome or
2 antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a
3 supernumerary gene insert.

1 52. The chimeric PIV of claim 51, which is selected from rBHPIV3-G1 or
2 rB/HPIV3-F1.

1 53. The chimeric PIV of claim 49, wherein one or more HPIV1 HN
2 and/or F gene(s) or genome segment(s) encoding one or more immunogenic domain(s),
3 fragment(s) or epitope(s) thereof are incorporated within the partial or complete bovine
4 genome or antigenome to form the vector genome or antigenome, which is further modified

5 by incorporation of one or more HPIV2 HN and/or F gene(s) or genome segment(s)
6 encoding one or more immunogenic domain(s), fragment(s) or epitope(s) thereof to form the
7 chimeric genome or antigenome which expresses protective antigen(s) from both HPIV1 and
8 HPIV2.

1 54. The chimeric PIV of claim 53, which is selected from rB/HPIV3.1-2F;
2 rB/HPIV3.1-2HN; or rB/HPIV3.1-2F,2HN.

3 55. The chimeric PIV of claim 1, wherein the vector genome or
4 antigenome is modified to encode a chimeric glycoprotein incorporating one or more
heterologous antigenic domains, fragments, or epitopes of a heterologous PIV or non-PIV
pathogen to form the chimeric genome or antigenome.

1 56. The chimeric PIV of claim 55, wherein the vector genome or
2 antigenome is modified to encode a chimeric glycoprotein incorporating one or more
3 antigenic domains, fragments, or epitopes from a second, antigenically distinct PIV to form
4 the chimeric genome or antigenome.

1 57. The chimeric PIV of claim 55, wherein the chimeric genome or
2 antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or
3 epitopes from two or more HPIVs.

1 58. The chimeric PIV of claim 55, wherein the heterologous genome
2 segment encodes a glycoprotein ectodomain which is substituted for a corresponding
3 glycoprotein ectodomain in the vector genome or antigenome.

1 59. The chimeric PIV of claim 55, wherein one or more heterologous
2 genome segment(s) of a second, antigenically distinct HPIV encoding said one or more
3 antigenic domains, fragments, or epitopes is/are substituted within a HPIV vector genome or
4 antigenome to encode said chimeric glycoprotein.

1 60. The chimeric PIV of claim 55, wherein heterologous genome
2 segments encoding both a glycoprotein ectodomain and transmembrane region are
3 substituted for counterpart glycoprotein ecto- and transmembrane domains in the vector
4 genome or antigenome.

1 61. The chimeric PIV of claim 55, wherein said chimeric glycoprotein is
2 selected from HPIV HN or F glycoproteins.

1 62. The chimeric PIV of claim 56, wherein the PIV vector genome or
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct
3 PIV is selected from HPIV1 or HPIV2.

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1 63. The chimeric PIV of claim 62, wherein the HPIV vector genome or
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct
3 HPIV is HPIV2.

1 64. The chimeric PIV of claim 63, wherein one or more glycoprotein
2 ectodomain(s) of HPIV2 is/are substituted for one or more corresponding glycoprotein
3 ectodomain(s) in the HPIV3 vector genome or antigenome.

1 65. The chimeric PIV of claim 64, wherein both glycoprotein
2 ectodomain(s) of HPIV2 HN and F glycoproteins are substituted for corresponding HN and
3 F glycoprotein ectodomains in the HPIV3 vector genome or antigenome.

1 66. The chimeric PIV of claim 65, which is rPIV3-2TM.

1 67. The chimeric PIV of claim 55, which is further modified to
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3
3 JS *cp45*.

1 68. The chimeric PIV of claim 55, which is rPIV3-2TM_{cp45}

1 69. The chimeric PIV of claim 55, wherein PIV2 ectodomain and
2 transmembrane regions of one or both HN and/or F glycoproteins is/are fused to one or more
3 corresponding PIV3 cytoplasmic tail region(s).

1 70. The chimeric PIV of claim 69, wherein ectodomain and
2 transmembrane regions of both PIV2 HN and F glycoproteins are fused to corresponding
3 PIV3 HN and F cytoplasmic tail regions.

1 71. The chimeric PIV of claim 70, which is rPIV3-2CT.

1 72. The chimeric PIV of claim 71, which is further modified to
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3
3 JS *cp45*.

1 73. The chimeric PIV of claim 72, which is rPIV3-2CT*cp45*.

1 74. The chimeric PIV of claim 55, which is further modified to
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3
3 JS *cp45* selected from mutations specifying an amino acid substitution in the L protein at a
4 position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a
5 position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C protein at a position
6 corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the
7 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or
8 a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS
9 *cp45*

1 75. The chimeric PIV of claim 55, wherein a plurality of heterologous
2 genes or genome segments encoding antigenic determinants of multiple heterologous PIVs
3 are added to or incorporated within the partial or complete HPIV vector genome or
4 antigenome.

1 76. The chimeric PIV of claim 75, wherein said plurality of heterologous
2 genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 and
3 are added to or incorporated within a partial or complete HPIV3 vector genome or
4 antigenome.

1 77. The chimeric PIV of claim 55, wherein the chimeric PIV genome or
2 antigenome is attenuated by addition or incorporation of one or more gene(s) or genome
3 segment(s) from a bovine PIV3 (BPIV3).

1 78. The chimeric PIV of claim 55, wherein the chimeric genome or
2 antigenome is modified by introduction of an attenuating mutation involving an amino acid
3 substitution of phenylalanine at position 456 of the HPIV3 L protein.

1 79. The chimeric PIV of claim 78, wherein phenylalanine at position 456
2 of the HPIV3 L protein is substituted by leucine.

1 80. The chimeric PIV of claim 55, wherein the chimeric genome or
2 antigenome incorporates one or more heterologous gene(s) or genome segment(s) encoding
3 one or more antigenic determinants from respiratory syncytial virus (RSV) or measles virus.

1 81. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome is modified by addition or substitution of one or more heterologous gene(s) or
3 genome segment(s) that confer increased genetic stability or that alter attenuation,
4 reactogenicity *in vivo*, or growth in culture of the chimeric virus.

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1 82. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome is modified by introduction of one or more attenuating mutations identified in a
3 biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA
4 virus.

1 83. The chimeric PIV of claim 82, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 present within PIV3 JS *cp45*.

1 84. The chimeric PIV of claim 82, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 specifying an amino acid substitution in the L protein at a position corresponding to Tyr₉₄₂,
4 Leu₉₉₂, or Thr₁₅₅₈ of in JS *cp45*; in the N protein at a position corresponding to residues Val₉₆
5 or Ser₃₈₉ of JS *cp45*, in the C protein at a position corresponding to Ile₉₆ of JS *cp45*, in the F
6 protein at a position corresponding to residues Ile₄₂₀ or Ala₄₅₀ of JS *cp45*, in the HN protein
7 at a position corresponding to residue Val₃₈₄ of JS *cp45*, a nucleotide substitution a 3' leader
8 sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of
9 JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to
10 nucleotide 62 of JS *cp45*.

1 85. The chimeric PIV of claim 82, wherein the chimeric genome or
2 antigenome incorporates attenuating mutations from different biologically derived mutant
3 PIVs or other mutant nonsegmented negative stranded RNA virus.

1 86. The chimeric PIV of claim 82, wherein the chimeric genome or
2 antigenome incorporates an attenuating mutation at an amino acid position corresponding to

3 an amino acid position of an attenuating mutation identified in a heterologous, mutant
4 negative stranded RNA virus.

1 87. The chimeric PIV of claim 86, wherein said attenuating mutation
2 comprises an amino acid substitution of phenylalanine at position 456 of the HPIV3 L
3 protein.

1 88. The chimeric PIV of claim 87, wherein phenylalanine at position 456
2 of the HPIV3 L protein is substituted by leucine.

1 89. The chimeric PIV of claim 82, wherein the chimeric genome or
2 antigenome includes at least one attenuating mutation stabilized by multiple nucleotide
3 changes in a codon specifying the mutation.

1 90. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome comprises an additional nucleotide modification specifying a phenotypic change
3 selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-
4 adaptation, plaque size, host-range restriction, or a change in immunogenicity.

1 91. The chimeric PIV of claim 90, wherein the additional nucleotide
2 modification alters one or more PIV N, P, C, D, V, M, F, HN and/or L genes and/or a 3'
3 leader, 5' trailer, and/or intergenic region within the vector genome or antigenome or within
4 the heterologous gene(s) or gene segment(s).

1 92. The chimeric PIV of claim 91, wherein one or more PIV gene(s) is
2 deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in
3 an RNA editing site, by a frameshift mutation, by a mutation that alters an amino acid
4 specified by an initiation codon, or by introduction of one or more stop codons in an open
5 reading frame (ORF) of the gene.

1 93. The chimeric PIV of claim 92, wherein the additional nucleotide
2 modification comprises a partial or complete deletion of one or more C, D or V ORF(s) or
3 one or more nucleotide change(s) that reduces or ablates expression of said one or more C, D
4 or V ORF(s).

1 94. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome is further modified to encode a cytokine.

1 95. The chimeric PIV of claim 1, which incorporates a heterologous gene
2 or genome segment from respiratory syncytial virus (RSV).

1 96. The chimeric PIV of claim 95, wherein the heterologous gene or
2 genome segment encodes RSV F and/or G glycoprotein(s) or immunogenic domain(s),
3 fragment(s), or epitope(s) thereof.

1 97. The chimeric PIV of claim 1 which is a virus.

1 ~~98. The chimeric PIV of claim 1 which is a subviral particle.~~

1 99. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 1 combined with a
4 physiologically acceptable carrier.

1 100. The method of claim 99, wherein the chimeric PIV is administered in
2 a dose of 10^3 to 10^7 PFU.

1 101. The method of claim 99, wherein the chimeric PIV is administered to
2 the upper respiratory tract.

1 102. The method of claim 99, wherein the chimeric PIV is administered by
2 spray, droplet or aerosol.

1 103. The method of claim 99, wherein the vector genome or antigenome is
2 of human PIV3 (HPIV3) and the chimeric PIV elicits an immune response against HPIV1
3 and/or HPIV2.

1 104. The method of claim 99, wherein the chimeric PIV elicits a
2 polyspecific immune response against multiple human PIVs and/or against a human PIV and
3 a non-PIV pathogen.

1 105. The method of claim 99, wherein the vector genome or antigenome is
2 a partial or complete human PIV (HPIV) genome or antigenome and the heterologous
3 pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial
4 viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency

5 ~~viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,~~
6 ~~filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.~~

1 106. The method of claim 99, wherein the chimeric PIV elicits a
2 polyspecific immune response against a human PIV (HPIV) and measles virus.

1 107. The method of claim 106, wherein the chimeric PIV elicits a
2 polyspecific immune response against HPIV3 and measles virus.

1 108. The method of claim 99, wherein a first, chimeric PIV according to
2 claim 1 and a second PIV are administered sequentially or simultaneously to elicit a
3 polyspecific immune response.

1 109. The method of claim 108, wherein the second PIV is a second,
2 chimeric PIV according to claim 1.

1 110. The method of claim 108, wherein the first, chimeric PIV and second
2 PIV are administered simultaneously in a mixture.

1 111. The method of claim 108, wherein the first, chimeric PIV and second
2 PIV are antigenically distinct variants of HPIV.

1 112. The method of claim 111, wherein the first, chimeric PIV comprises a
2 partial or complete HPIV3 genome or antigenome combined with one or more heterologous
3 gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a different
4 PIV.

1 113. The method of claim 111, wherein the first, chimeric PIV and second
2 PIV each incorporate one or more heterologous gene(s) or genome segment(s) encoding one
3 or more antigenic determinant(s) of a non-PIV pathogen.

1 114. The method of claim 113, wherein the first and second chimeric PIV
2 incorporate one or more heterologous gene(s) or genome segment(s) encoding one or more
3 antigenic determinant(s) of the same non-PIV pathogen.

1 115. A method for sequential immunization to stimulate the immune
2 system of an individual to induce protection against multiple pathogens comprising
3 administering to a newborn to 4 month old infant an immunologically sufficient amount of a

4 first attenuated chimeric HPIV expressing an antigenic determinant of a non-PIV pathogen
5 and one or more antigenic determinants of HPIV3 and subsequently administering an
6 immunologically sufficient amount of a second attenuated chimeric HPIV expressing an
7 antigenic determinant of a non-PIV pathogen and one or more antigenic determinants of
8 HPIV1 or HPIV2.

1 116. The method for sequential immunization of claim 115, wherein the
2 first attenuated chimeric HPIV is an HPIV3 expressing a measles virus antigenic determinant
3 and wherein the second attenuated chimeric HPIV is a PIV3-1 chimeric virus expressing a
4 measles virus antigenic determinant and incorporating one or more attenuating mutations of
5 HPIV3 JS *cp45*.

1 117. The method for sequential immunization of claim 115, wherein
2 following the first vaccination, the vaccinee elicits a primary antibody response against both
3 PIV3 and the non-PIV pathogen, but not HPIV1 or HPIV2, and upon secondary
4 immunization the vaccinee is readily infected with the second attenuated HPIV and develops
5 both a primary antibody response to HPIV1 or HPIV2 and a high titered secondary antibody
6 response against the non-PIV pathogen.

1 118. The method for sequential immunization of claim 115, wherein the
2 first chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV
3 elicits an immune response against HPIV1 or HPIV2, and wherein both the first and second
4 chimeric PIVs elicit an immune response against measles or RSV.

1 119. The method for sequential immunization of claim 115, wherein the
2 non-PIV pathogen is selected from measles virus, subgroup A and subgroup B respiratory
3 syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and type 2 human
4 immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein
5 Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1 120. The method for sequential immunization of claim 115, wherein the
2 second chimeric PIV comprises a partial or complete HPIV3 vector genome or antigenome
3 combined with one or more gene(s) or genome segment(s) encoding one or more HPIV1
4 and/or HPIV2 HN and/or F glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s)
5 thereof.

1 121. ~~The method for sequential immunization of claim 115, wherein the~~
2 partial or complete vector genome or antigenome of the first, chimeric PIV incorporates at
3 least one and up to a full complement of attenuating mutations present within HPIV3 JS
4 cp45 selected from mutations specifying an amino acid substitution in the L protein at a
5 position corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a
6 position corresponding to residues Val96 or Ser389 of JS cp45, in the C protein at a position
7 corresponding to Ile96 of JS cp45, a nucleotide substitution a 3' leader sequence of the
8 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or
9 a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS
10 cp45.

1 122. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in a
3 physiologically acceptable carrier.

1 123. The immunogenic composition of claim 122, formulated in a dose of
2 10^3 to 10^7 PFU.

1 124. The immunogenic composition of claim 122, formulated for
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1 125. The immunogenic composition of claim 122, wherein the chimeric
2 PIV elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2
3 and HPIV3.

1 126. The immunogenic composition of claim 122, wherein the chimeric
2 PIV elicits an immune response against HPIV3 and another virus selected from HPIV1 and
3 HPIV2.

1 127. The immunogenic composition of claim 122, wherein the chimeric
2 PIV elicits a polyspecific immune response against one or more HPIVs and a heterologous
3 pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial
4 viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency
5 viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,
6 filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1 128. The immunogenic composition of claim 127, wherein the chimeric
2 PIV elicits a polyspecific immune response against HPIV3 and measles or respiratory
3 syncytial virus

1 129. The immunogenic composition of claim 122, further comprising a
2 second, chimeric PIV according to claim 1.

1 130. The immunogenic composition of claim 129, wherein the first and
2 second chimeric PIVs are antigenically distinct variants of HPIV and bear the same or
3 different heterologous antigenic determinant(s).

1 131. The immunogenic composition of claim 129, wherein the first
2 chimeric PIV comprises a partial or complete HPIV3 genome or antigenome combined with
3 one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic
4 determinant(s) of a non-PIV heterologous pathogen.

1 132. The immunogenic composition of claim 129, wherein the second
2 chimeric PIV incorporates one or more heterologous gene(s) or genome segment(s) encoding
3 one or more antigenic determinant(s) of the same non-PIV heterologous pathogen.

1 133. The immunogenic composition of claim 129, wherein the first
2 chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits
3 an immune response against HPIV1 or HPIV2, and wherein both the first and second
4 chimeric PIVs elicit an immune response against the non-PIV pathogen.

1 134. The immunogenic composition of claim 129, wherein the
2 heterologous pathogen is selected from measles virus, subgroup A and subgroup B
3 respiratory syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and
4 type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies
5 virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza
6 viruses.

1 135. The immunogenic composition of claim 129, wherein the
2 heterologous pathogen is selected from measles virus or RSV.

1 136. The immunogenic composition of claim 129, wherein the second
2 chimeric PIV comprises a partial HPIV3 vector genome or antigenome combined with one

3 or more HPIV1 gene(s) or genome segment(s) encoding one or more antigenic determinants
4 of HPIV1 HN and/or F glycoproteins.

1 137. The immunogenic composition of claim 129, wherein the second
2 chimeric PIV compresses a partial or complete HPIV3 vector genome or antigenome
3 combined with one or more gene(s) or genome segment(s) encoding one or more HPIV2 HN
4 and/or F glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s) thereof.

1 138. An isolated polynucleotide comprising a chimeric PIV genome or
2 antigenome which includes a partial or complete PIV vector genome or antigenome
3 combined with one or more heterologous gene(s) or genome segment(s) encoding one or
4 more antigenic determinant(s) of one or more heterologous pathogen(s) to form a chimeric
5 PIV genome or antigenome.

1 139. The isolated polynucleotide of claim 138, wherein said one or more
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are
3 added adjacent to or within a noncoding region of the partial or complete PIV vector genome
4 or antigenome.

1 140. The isolated polynucleotide of claim 138, wherein said one or more
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are
3 substituted for one or more counterpart gene(s) or genome segment(s) in a partial PIV vector
4 genome or antigenome.

1 141. The isolated polynucleotide of claim 138, wherein said one or more
2 heterologous pathogens is a heterologous PIV and said heterologous gene(s) or genome
3 segment(s) encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or
4 immunogenic fragment(s), domain(s), or epitope(s) thereof.

1 142. The isolated polynucleotide of claim 138, wherein the vector genome
2 or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the
3 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of
4 one or more heterologous PIV(s).

1 143 The isolated polynucleotide of claim 142, wherein the vector genome
2 or antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous

3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of HPIV 1 and/or
4 HPIV2.

1 144. The isolated polynucleotide of claim 138, wherein the vector genome
2 or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the
3 heterologous pathogen is selected from measles virus, subgroup A and subgroup B
4 respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2
5 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus,
6 Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza
7 viruses.

1 145. The isolated polynucleotide of claim 144, wherein said one or more
2 heterologous antigenic determinant(s) is/are selected from measles virus HA and F proteins,
3 subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps
4 virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2 human
5 immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus gB, gC,
6 gD, E, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus
7 gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and NS1 proteins, and
8 alphavirus E protein, and antigenic domains, fragments and epitopes thereof.

1 146. The isolated polynucleotide of claim 138, wherein the vector genome
2 or antigenome is a partial or complete HPIV3 genome or antigenome or a chimeric HPIV
3 genome or antigenome comprising a partial or complete HPIV3 genome or antigenome
4 having one or more gene(s) or genome segment(s) encoding one or more antigenic
5 determinant(s) of a heterologous HPIV added or incorporated therein.

1 147. The isolated polynucleotide of claim 146, wherein the heterologous
2 pathogen is measles virus and the heterologous antigenic determinant(s) is/are selected from
3 the measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 148. The isolated polynucleotide of claim 147, wherein a transcription unit
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or
3 incorporated within a HPIV3 vector genome or antigenome.

1 149. The isolated polynucleotide of claim 147, wherein a transcription unit
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or

3 incorporated within a HPIV3-1 vector genome or antigenome having both the HPIV3 HN
4 and F ORFs substituted by the HN and F ORFs of HPIV1.

1 150. The isolated polynucleotide of claim 138, wherein the partial or
2 complete PIV vector genome or antigenome is combined with one or more supernumerary
3 heterologous gene(s) or genome segment(s) to form the chimeric PIV genome or
4 antigenome.

1 151. The isolated polynucleotide of claim 150, wherein the vector genome
2 or antigenome is a partial or complete HPIV3 genome or antigenome and said one or more
3 supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN,
4 HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene
5 unit.

1 152. The isolated polynucleotide of claim 138, wherein one, two or all of
2 the HPIV1 HN, HPIV2 HN, and measles virus HA ORFs are added to the vector genome or
3 antigenome.

1 153. The isolated polynucleotide of claim 138, wherein one or more of the
2 HPIV1 HN and HPIV2 HN genes and a 3918-nt GU insert is/are added are added to the
3 vector genome or antigenome.

1 154. The isolated polynucleotide of claim 150, wherein said one or more
2 supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign
3 sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to
4 the wild-type HPIV3 genome length of 15,462 nt.

1 155. The isolated polynucleotide of claim 138, wherein the vector genome
2 or antigenome is a human-bovine chimeric PIV genome or antigenome.

1 156. The isolated polynucleotide of claim 155, wherein the human-bovine
2 chimeric vector genome or antigenome is combined with one or more heterologous gene(s)
3 or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous
4 pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial
5 viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency
6 viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,
7 filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses

1 157. The isolated polynucleotide of claim 156, wherein the vector genome
2 or antigenome comprises a partial or complete HPIV genome or antigenome combined with
3 one or more heterologous gene(s) or genome segment(s) from a BPIV.

1 158. The isolated polynucleotide of claim 157, wherein a transcription unit
2 comprising an open reading frame (ORF) of a BPIV3/N ORF is substituted in the vector
3 genome or antigenome for a corresponding N ORF of a HPIV3 vector genome.

1 159. The isolated polynucleotide of claim 158, wherein the vector genome
2 or antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

1 160. The isolated polynucleotide of claim 138, wherein the vector genome
2 or antigenome comprises a partial or complete BPIV genome or antigenome combined with
3 one or more heterologous gene(s) or genome segment(s) from a HPIV.

1 161. The isolated polynucleotide of claim 160, wherein one or more HPIV
2 gene(s) or genome segment(s) encoding HN and/or F glycoproteins or one or more
3 immunogenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated
4 within the partial or complete bovine genome or antigenome to form the vector genome or
5 antigenome.

1 162. The isolated polynucleotide of claim 161, wherein both HPIV3 genes
2 encoding HN and F glycoproteins are substituted for corresponding BPIV3 HN and F genes
3 to form the vector genome or antigenome.

1 163. The isolated polynucleotide of claim 162, wherein the vector genome
2 or antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a
3 supernumerary gene insert.

1 164. The isolated polynucleotide of claim 138, wherein the chimeric
2 genome or antigenome encodes a chimeric glycoprotein having antigenic domains,
3 fragments, or epitopes from both a human PIV (HPIV) and a heterologous pathogen.

1 165. The isolated polynucleotide of claim 164, wherein the chimeric
2 genome or antigenome encodes a chimeric glycoprotein having antigenic domains,
3 fragments, or epitopes from two or more different PIVs.

1 166. The isolated polynucleotide of claim 138, wherein the chimeric
2 genome or antigenome is modified by introduction of one or more attenuating mutations
3 identified in a biologically derived mutant PIV or other mutant nonsegmented negative
4 stranded RNA virus.

1 167. The isolated polynucleotide of claim 138, wherein, the chimeric
2 genome or antigenome incorporates at least one and up to a full complement of attenuating
3 mutations present within PIV3 JS *cp45*.

1 168. The isolated polynucleotide of claim 138, wherein the chimeric
2 genome or antigenome incorporates an attenuating mutation from a heterologous
3 nonsegmented negative stranded RNA virus.

1 169. The isolated polynucleotide of claim 138, wherein the chimeric
2 genome or antigenome comprises an additional nucleotide modification specifying a
3 phenotypic change selected from a change in growth characteristics, attenuation,
4 temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in
5 immunogenicity.

1 170. The isolated polynucleotide of claim 138, wherein the additional
2 nucleotide modification alters one or more PIV N, P, C, D, V, M, F, HN and/or L genes
3 and/or a 3' leader, 5' trailer, and/or intergenic region within the vector genome or
4 antigenome or within the heterologous gene(s) or gene segment(s).

1 171. The isolated polynucleotide of claim 138, wherein one or more PIV
2 gene(s) is deleted in whole or in part or expression of the gene(s) is reduced or ablated by a
3 mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters an amino
4 acid specified by an initiation codon, or by introduction of one or more stop codons in an
5 open reading frame (ORF) of the gene.

1 172. A method for producing an infectious attenuated chimeric PIV particle
2 from one or more isolated polynucleotide molecules encoding said PIV, comprising:

3 expressing in a cell or cell-free lysate an expression vector comprising an
4 isolated polynucleotide comprising a partial or complete PIV vector genome or antigenome
5 of a human or bovine PIV combined with one or more heterologous gene(s) or genome

6 segment(s) encoding one or more antigenic determinant(s) of one or more heterologous
7 pathogen(s) to form a chimeric PIV genome or antigenome, and PIV N, P, and L proteins.

1 173. The method of claim 172, wherein the chimeric PIV genome or
2 antigenome and the N, P, and L proteins are expressed by two or more different expression
3 vectors.

1 174. An expression vector comprising an operably linked transcriptional
2 promoter, a polynucleotide sequence which includes a partial or complete PIV vector
3 genome or antigenome of a human or bovine PIV combined with one or more heterologous
4 gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more
5 heterologous pathogen(s) to form a chimeric PIV genome or antigenome, and a
6 transcriptional terminator.

1 175. An isolated infectious recombinant parainfluenza virus (PIV)
2 comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large
3 polymerase protein (L), and a PIV genome or antigenome having a polynucleotide insertion
4 of between 150 nucleotides (nts) and 4,000 nucleotides in length in a non-coding region
5 (NCR) of the genome or antigenome or as a separate gene unit (GU), said polynucleotide
6 insertion lacking a complete open reading frame (ORF) and specifying an attenuated
7 phenotype in said recombinant PIV.

1 176. The recombinant PIV of claim 175, wherein said polynucleotide insert
2 is introduced into the PIV genome or antigenome in a reverse, non-sense orientation
3 whereby the insert does not encode protein.

1 177. The recombinant PIV of claim 175, wherein said polynucleotide insert
2 is approximately 2,000 nts or greater in length.

1 178. The recombinant PIV of claim 175, wherein said polynucleotide insert
2 is approximately 3,000 nts or greater in length.

1 179. The recombinant PIV of claim 175, wherein said recombinant PIV
2 replicates efficiently *in vitro* and exhibits an attenuated phenotype *in vivo*.